

Monitoring Bloom Dynamics of a Common Coastal Bioluminescent Ctenophore

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LONG-TERM GOALS

The long-term objective is to develop predictive models of bioluminescence potential in the coastal zone environment.

OBJECTIVES

In the coastal zone, watershed run-off and discharge of submarine ground-water can profoundly impact growth conditions of bioluminescent plankton on very short space and time scales. Bioluminescent blooms include dinoflagellate red tides, which are occurring more frequently, lasting longer and extending further off shore due to excessive nutrient loading from land-based run-off. Bioluminescent blooms of the carnivorous ctenophore *Mnemiopsis leidyi* that may be either seasonal or event driven can also develop on remarkably short time scales (Kremer, 1994) and also appear to be on the increase (Sullivan et al., 2001).

The Ocean Research & Conservation Association's (ORCA) objective is to improve understanding of the conditions leading to bioluminescent blooms in coastal waters such that forecasting is possible. Achieving this requires more frequent, automated sampling of greater spatial density, a reduction in dependence upon hand sampling, and automating all aspects of the data collection and analysis processes.

APPROACH

Increasing the frequency of field sampling and removing the bias introduced by fair-weather sampling requires automation. Achieving greater spatial density of measurements in a sustainable manner requires reducing the cost of sensor systems through component-level integration. New technologies, such as ORCA's bathyphotometer to measure bioluminescence, are necessary to automate biological sample collection and processing. When hand sampling is still necessary, the processing should make use of automated methods for processing. ORCA's approach to monitoring bloom dynamics is to supplement the ORCA Kilroy network with low-cost, autonomous bathyphotometers, using the intensity of bioluminescence as an estimator of abundance and the flash kinetics to assist in distinguishing organisms.

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The Kilroy network consists of remote, autonomous, pile-mounted stations that communicate field measurements via cellular Internet access to a central database. Each station, at minimum, consists of an above-water cellular/EI-485 gateway called Kilroy's Voice, a solar panel, charge-controller, and reserve battery – all of which support additional proprietary and off-the-shelf smart sensor systems daisy-chained together on a sensor string. Devices on the sensor string share power and a multi-drop EI-485 communications link to Kilroy's Voice. Kilroy's Voice also logs time and position using an on-board GPS, measures barometric pressure, monitors reserve battery charge, and synchronizes clocks on devices on the sensor string. On each sensor string, at minimum, is a single Kilroy sensor suite that monitors flow-speed, flow-direction, water temperature, water level, wave-height, and wave-period. Additional sensor systems connect to the Kilroy network via the sensor string. Stations are equipped with different instrumentation system sets, which may include a bathyphotometer to measure bioluminescence intensity, an Aanderaa salinometer, Campbell Scientific CR1000 data logger, a Texas Electronics TE-525 or Hydrological Services TB4 rain gauge, an RM Young Wind Sentry anemometer and wind vane, an Apogee Instruments pyranometer, and a shielded air temperature sensor. Figure 1 shows the Google Maps based web interface to the latest Kilroy sensor suite measurements from the database.



Figure 1 Web interface to latest Kilroy measurements from the database. Stations are all on pilings of docks of volunteers in the Choptank River in the Chesapeake Bay, and are located where indicated by flow vectors. Flow vectors point in direction of most recent flow measurements and are color-coded based on temperature. Mousing over any vector pulls up most recent measurements from the corresponding station, as shown above for station number C12. Measurements shown on gauges to the right are selectable by the user.

Monitoring changes in mesozooplankton abundance and species composition has been considered to be critical for identifying ecological changes in marine ecosystems (Kane, 2009). Gelatinous zooplankton, such as *Mnemiopsis* sp., feed on mesozooplankton, with copepods being their main food source (Monteleone and Duguay, 1988). In an effort to identify correlations between ctenophore abundance and that of mesozooplankton and to further provide a sample set which could be compared with bathyphotometer estimates of abundance through bioluminescence intensity, an ambitious sampling scheme of mesozooplankton was conducted. To increase the efficiency of processing the net-collected samples, they were processed using a Fluid Imaging Technologies FlowCAM imaging flow cytometer.

Graduate student Miranda Kerr, now at the Shedd Aquarium, had the responsibility of collecting and processing zooplankton samples and developed the protocols and techniques used to process the samples using the imaging flow cytometer. ORCA's engineering team, consisting of Ocean Engineer Tony Cimaglia, Instrumentation Engineer John Taylor, and Principal Engineer Dr. Eric Thosteson, was responsible for all sensor, power, telemetry, and database system development and testing, and for integration of new instruments into the Kilroy network. Managing Director Warren Falls and Field Technician Jerry Corsault made up the field maintenance and operations team. Florida Institute of Technology Meteorology Professors Dr. Stephen Lazarus and Michael Splitt with graduate student Forbes Tompkins, developed the meteorological data aggregation and visualization scripts and assisted in meteorological instrumentation selection. Peter Thosteson of Atomic Lightwave created the map-based web interface.

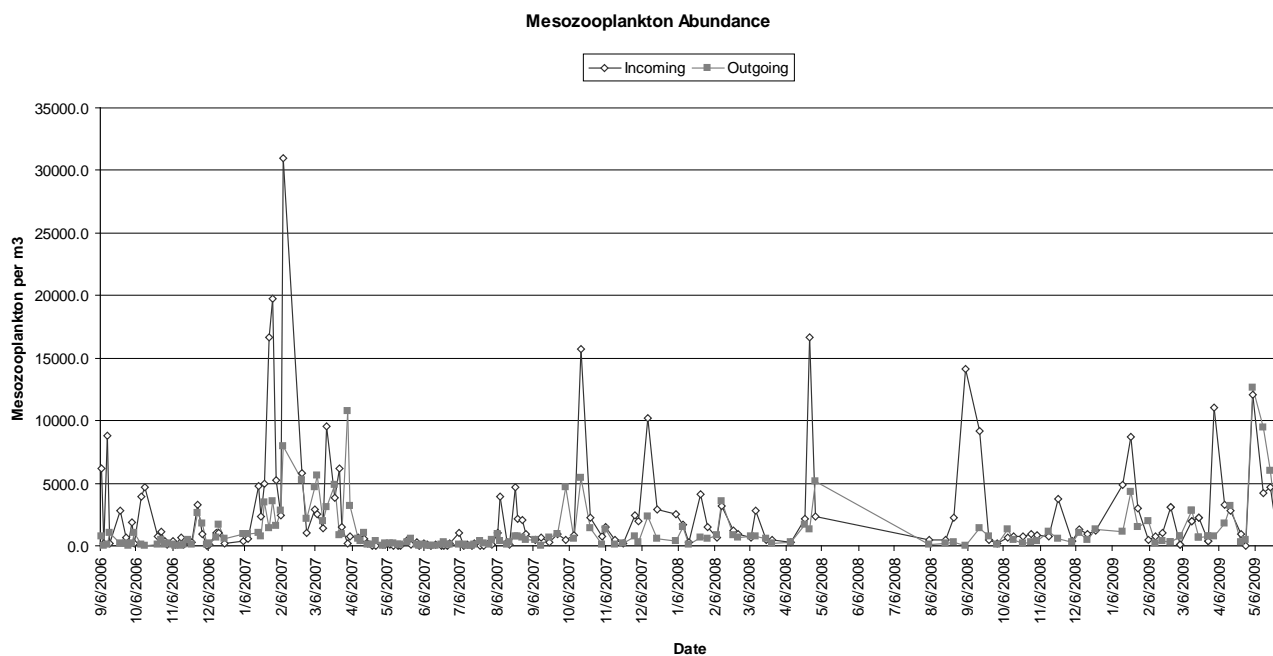


Figure 2 *Mesozooplankton abundance (individuals per cubic meter) on the incoming and outgoing tides from September 2006 to May 2009, collected at the Dockside Inn, Fort Pierce, FL. (Gap in data collection during June and July 2008)*

WORK COMPLETED

Mesozooplankton samples were collected, categorized, and counted from September 2006 until May 2009, and laboratory techniques using an imaging flow cytometer were developed to efficiently process these samples. Shown in Figure 2 are the zooplankton abundances measured weekly over this period. Ctenophores were also collected from a number of sites. A planktonkreisel was developed to increase the longevity of ctenophores collected from the field for use in laboratory studies.

The sensor systems comprising ORCA's Kilroy network were deployed at several sites, the largest yet being a 9-station deployment in the Choptank river in the Chesapeake (see Figure 1). A Kilroy sensor suite was deployed alongside a horizontal Teledyne RDI ADCP maintained by USGS in Jewfish Creek, just north of Key Largo in the Florida Keys for comparison of flow measurements.

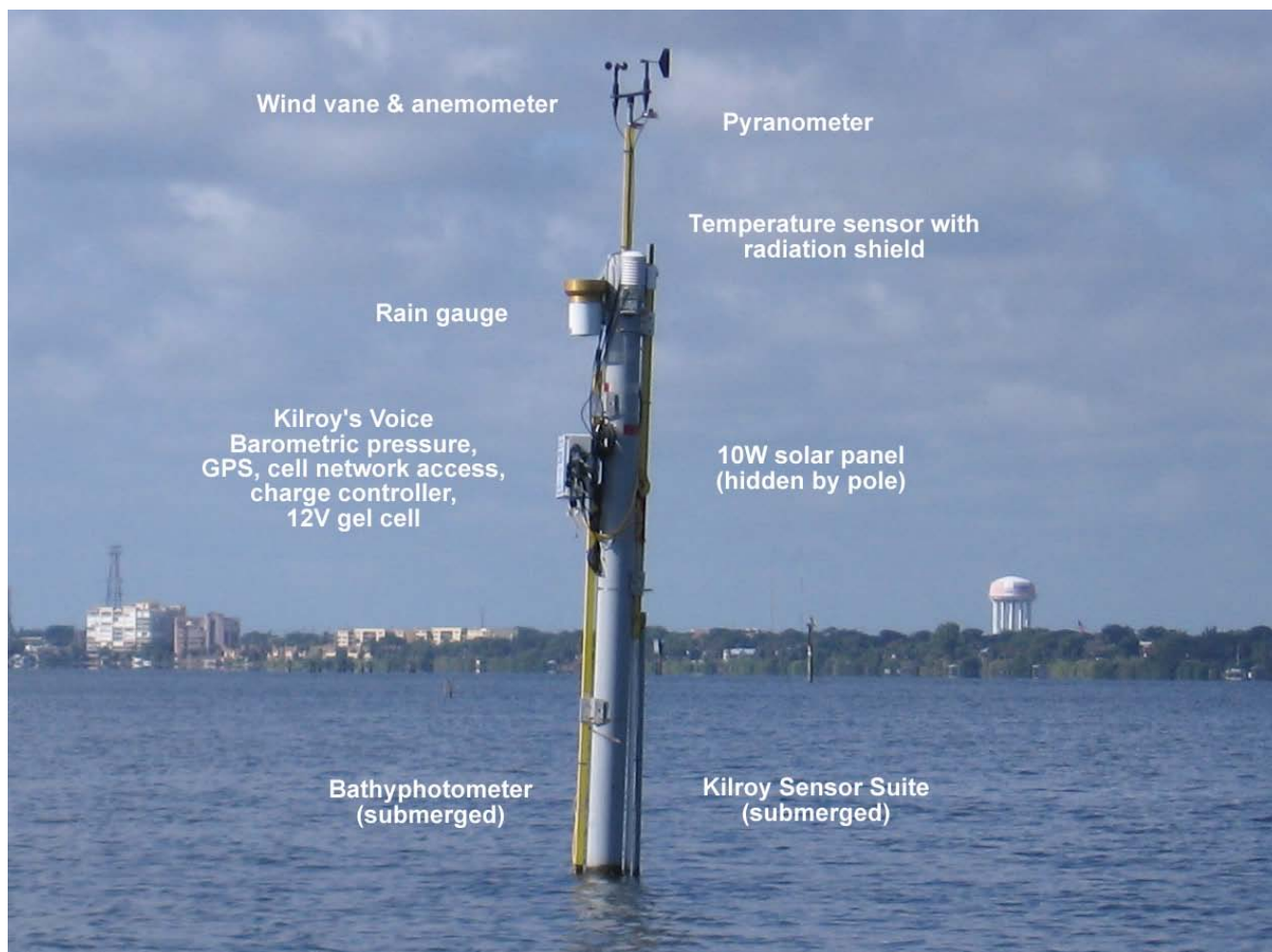


Figure 3 Kilroy Network station deployed west of Cocoa, Florida. Station consists of a complete meteorological station, Kilroy sensor suite, bathypotometer, Kilroy's Voice cellular Internet gateway, a 12 Ah, 12V gel cell battery, charge controller, and 10W solar panel.

Meteorological events may lead to bloom development, be it through seasonal increase in insolation, nutrient loading in run-off from precipitation events, or increased mixing due to wind and wave events

In conjunction with Florida Tech meteorologists, scripts were written to collect rainfall analysis and solar insolation products from NCEP, and generate regional maps for use in map overlays synchronous with measurements from the Kilroy network. Furthermore, a suite of meteorological sensors including a rain gauge, pyranometer, anemometer and wind vane, and a shielded temperature sensor were integrated into the Kilroy network. A site just west of the city of Cocoa including the Kilroy's Voice/Kilroy minimum setup along with a CR1000, anemometer and wind vane, shielded temperature sensor, pyranometer, rain gauge, and bathyphotometer is shown in Figure 3.

Results from laboratory tests of the bathyphotometer designed for use on the Kilroy network at the end of 2009 indicated that greater photosensitivity was required. Several modifications were made to improve sensitivity and increase deployment duration, and two additional bathyphotometers were built. Initial sensitivity improvements to the bathyphotometer included moving the photodetectors closer to the clear acrylic test section to reduce the optical path length and reducing integrator capacitance to increase transimpedance gain. A later improvement to sensitivity came when existing circuit boards were retrofitted with surface mount U.F.L. coaxial connectors to allow larger area photodiodes to be mounted above the small area originally allocated for photodiodes. The shielded connectors made this possible without compromising the design through introduction of additional noise. Additional laboratory and field testing then revealed that small op amp offset errors were masking the photodiode output values by dropping them below the minimum measureable analog level, so another modification was made to add a constant offset to the photodiode signals.

Field tests of the redesigned bathyphotometer began in May of 2010 and are still ongoing. The bathyphotometer follows a scheme of sampling hourly from 9 PM, EDT until 7 AM, EDT (1 AM, UTC – 11 AM, UTC). Each hour, after a one-minute sensor warm-up interval, the 10-elements of the photodiode array are simultaneously burst sampled through integrating transimpedance amplifiers with an integration period of 0.1 s. 64-bursts are collected in each record. The pump is then started and allowed to run for 10-seconds to allow the flow to stabilize. A second 64-burst record is then recorded from the array.

RESULTS

Protocols and techniques for mesozooplankton monitoring with net sampling and imaging flow cytometry were established. Mesozooplankton samples were collected on incoming and outgoing tides and filtered to analyze the mesozooplankton in the size range 100 to 850 μm . These samples were run through the imaging flow cytometer and images were classified into groups to genera.

Mesozooplankton samples were collected and enumerated from September 2006 to May 2009, with a break in sampling from May to July 2008 for equipment repairs. These data provide comparisons of seasonal differences, and differences in the mesozooplankton assemblage on the incoming and outgoing tide. The abundance of mesozooplankton varied significantly between the incoming and outgoing tides ($p < 0.001$). The mean abundance of mesozooplankton on the incoming tide (2298.2 individuals/ m^3) was twice as great as the mean abundance of mesozooplankton on the outgoing tide (1180.0 individuals/ m^3). In addition, the maximum abundance on the incoming tide (30957.1 individuals/ m^3) was three times greater than the maximum abundance of individuals on the outgoing tide (10791.5 individuals/ m^3). These data suggest that the ocean acts as a source of mesozooplankton for the IRL estuary. The zooplankton may not leave the estuary because they are consumed by predators such as *Mnemiopsis* sp., and juvenile fish species such as mullet (*Mugil* spp.), ladyfish (*Elops saurus*), and snook (*Centropomus undecimalis*) (Monteleone and Duguay 1988, Rey *et al.* 1991). The most abundant type of mesozooplankton was the copepod *Acartia tonsa*, a known prey

item of *Mnemiopsis* sp., representing 35.0% and 52.1% of the individuals on the incoming and outgoing tides respectively. Large variations in zooplankton abundance (Figure 2) did not correlate with any environmental variables nor with the presence or absence of *Mnemiopsis*, which demonstrated a highly patchy distribution and was not detected in the vicinity of the Kilroy bathyphotometer deployment. The strongest correlation was found between chlorophyll *a* concentrations and *A. tonsa* abundance. The complex advection patterns of a tidal estuary are a likely contributor to the extreme variability and patchiness which were observed.

Measurements of water level and flow provide critical information towards predictive models of bloom development, since hydrological transport modeling is required to separate unsteady variations in measured abundances due to reproduction and predation from variations due to advection of patches of organisms. To verify the ability of the inexpensive acoustic travel time current meter on the Kilroy sensor suite to make these critical measurements reliably, the Kilroy sensor suite's measurements of flow magnitude and direction were compared to those from the USGS maintained acoustic flow meter deployed in Jewfish Creek, just north of Key Largo. Results from almost three weeks of data collection at 30 minute intervals, shown in Figure 4, demonstrate that the measurements from the two sensor systems agree well.

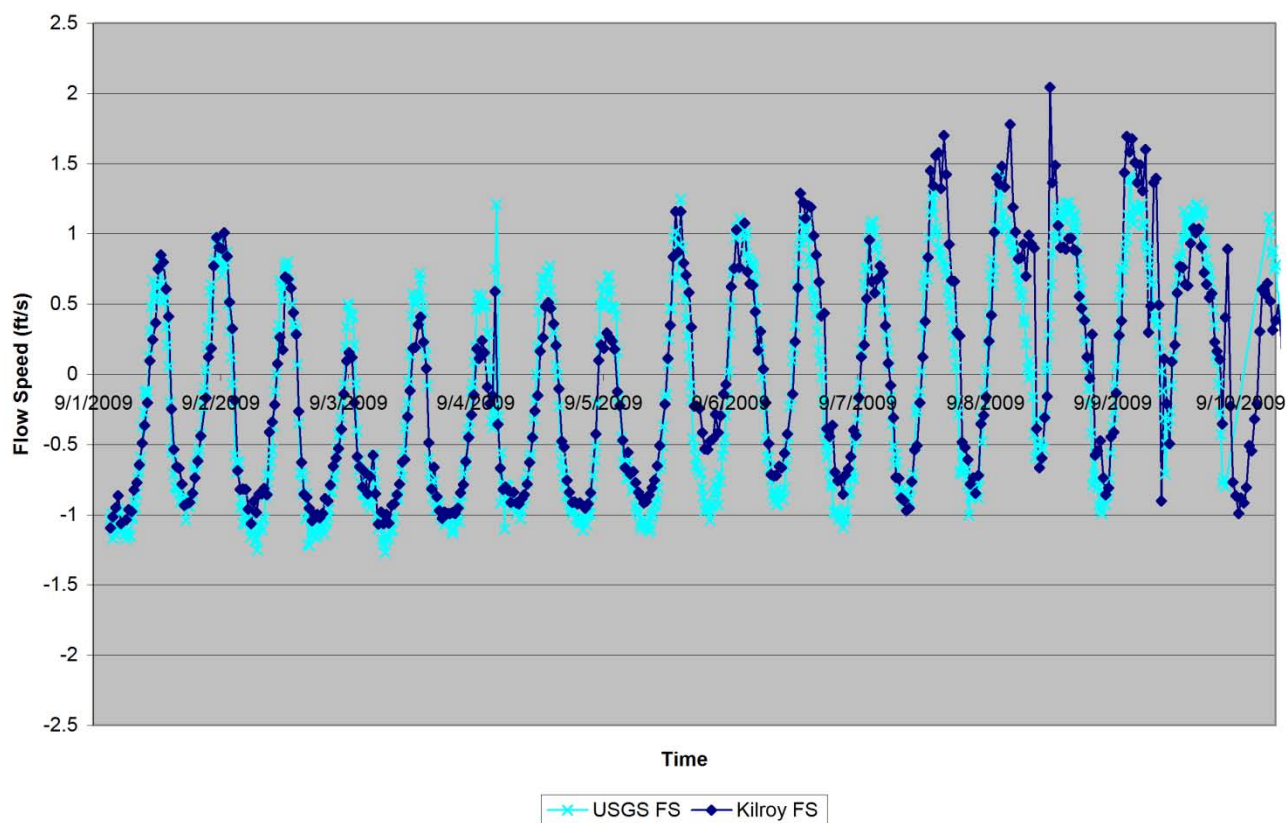


Figure 4 Comparison between measurements from a USGS maintained flow meter in Jewfish creek, north of Key Largo, Florida and collocated Kilroy acoustic travel time flow meter from Sept. 1-10, 2009. Light blue data points in background are from the USGS sensor, while darker blue points in the foreground are from the Kilroy acoustic flow meter.

Results from several week-long deployments of the bathyphotometer in the Indian River Lagoon, just west of Cocoa, Florida (the station is shown in Figure 3), produced light profiles that suggested the presence of bioluminescent organisms, but many of the resulting profiles were collected during daylight hours. Because bioluminescent dinoflagellates are photically inhibited, the measurements were unlikely a result of bioluminescence, but were more likely associated with drift in the photodiode measurements. It was decided to modify the bathyphotometer sampling scheme to that described earlier - first sampling from the photodiode array with the pump off and then sampling again soon thereafter, just long enough for the pump to stabilize to a consistent volumetric flow rate. This provides background measurements that are made within seconds before the mechanically excited bioluminescence intensity measurements are made. Additional field tests conducted from August 26, 2010 until September 26, 2010 show variations in bioluminescence intensity, shown in Figure 5, on the few photodiodes having positive offsets on that version of the bathyphotometer. The improvement in the signal characteristics achieved by producing the more recent background measurements is dramatic, but also revealed the need for still greater sensitivity and correction of the signal offset.

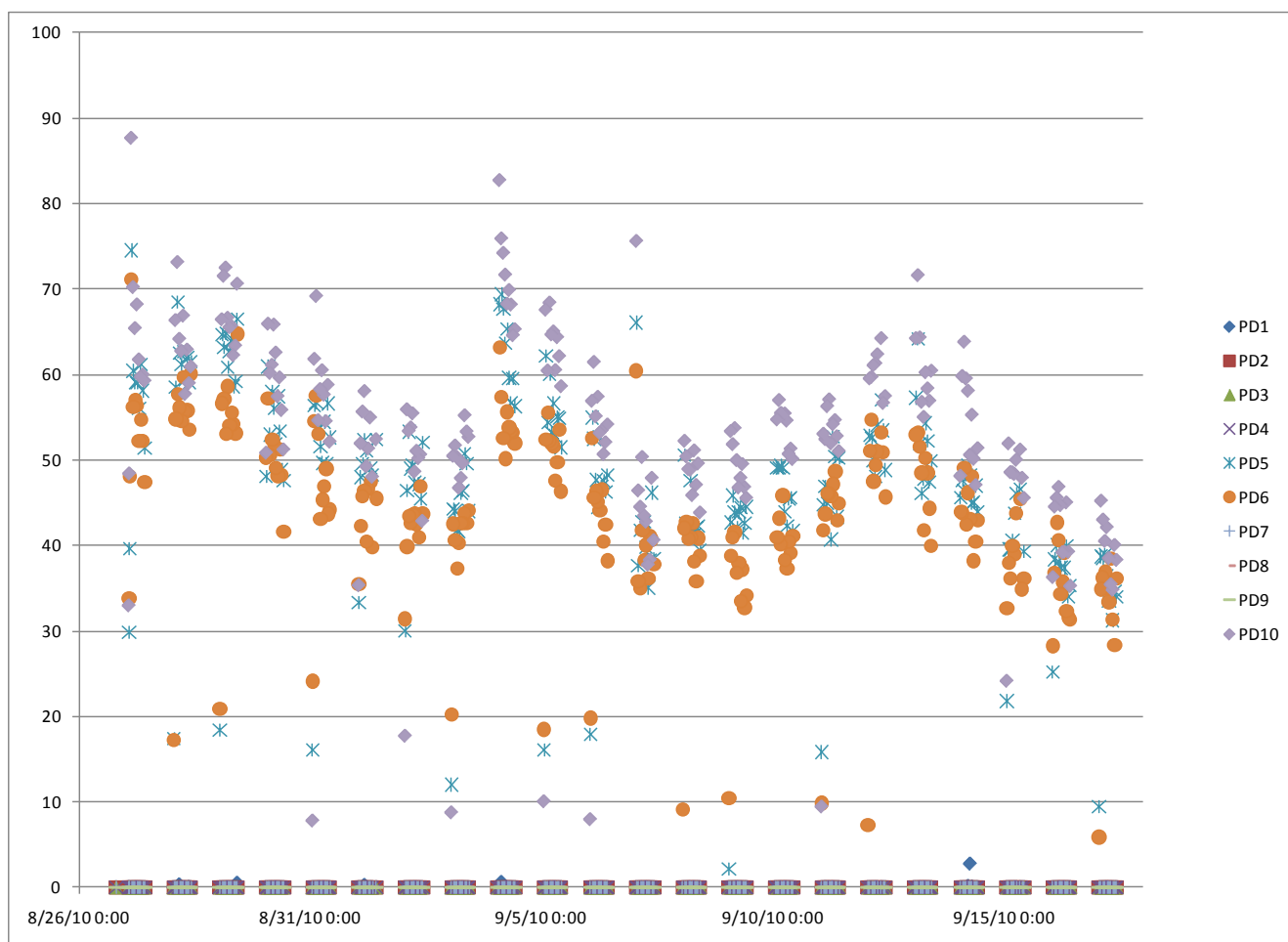


Figure 5 Variations in bioluminescence intensity from a less photosensitive version of the bathyphotometer. Light levels were only recorded on those photodiodes that, by chance, had positive offsets – keeping the signal levels within the measurement range.

Introduction of a voltage offset to the photodiode measurements and substitution of larger area photodiodes are modifications that were recently made, and the latest version of the bathyphotometer was tested in the laboratory using cultures of the dinoflagellates *Lingulodinium polyedrum* and *Pyrocystis fusiformis*. As hoped, the excitation response curves produced, one of which is shown in Figure 6, were similar in form to those produced by the HIDEX 3 (Davis et al., 2005). This is a highly significant accomplishment as it provides proof-of-concept that a solid-state detector system can replicate the HIDEX III capability of identification of bioluminescent populations by excitation response patterns. Still greater sensitivity is possible and will be tested in the near future through improved optical gain, higher resolution analog to digital conversion, and greater transimpedance gain.

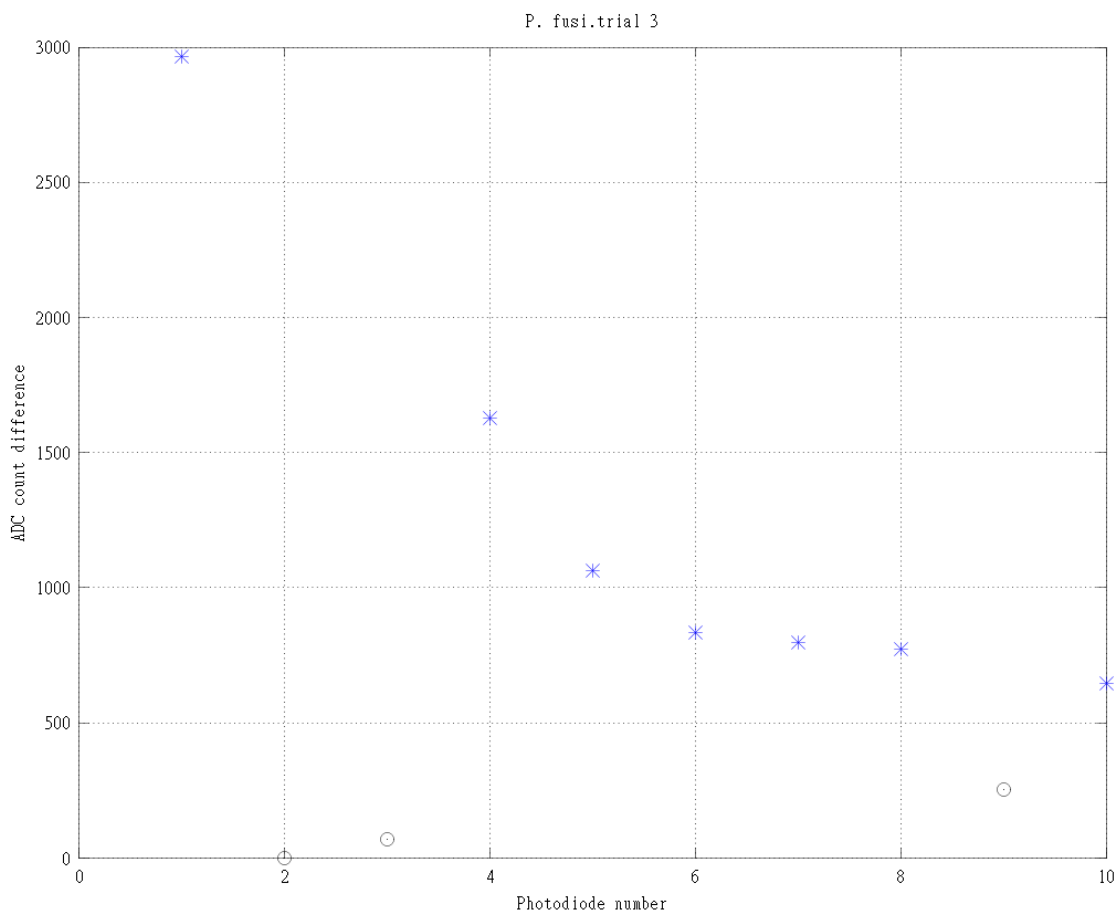


Figure 5 *Bioluminescence intensities from Pyrocystis fusiformis in laboratory tests of bathyphotometer. Photodiode number 1 is closest to the point of excitation. Circles indicate dysfunctional photodiodes.*

IMPACT/APPLICATIONS

More frequent and more rapidly developing jellyfish blooms, especially *Mnemiopsis leidyi* as well as Harmful Algal Blooms, increase the importance of more efficient and effective monitoring techniques. Because large blooms of *Mnemiopsis leidyi* have decimated fisheries, the factors giving rise to these blooms must be identified before they can be controlled or predicted. The impact of an unexpected

bioluminescent bloom on clandestine military operations could be devastating. The ability to predict bioluminescent blooms depends upon more frequent and dense measurements of the organisms and the factors contributing to the bloom, but collected with an efficiency only sustainable through automation.

To meet the need for a bioluminescent jellyfish monitoring and forecasting system, predictive models will depend upon dense networks of sensor systems capable of frequent, automated measurements of the variables governing bloom development. Just as the accuracy of weather forecasts rely upon sound measurements of initial conditions, so will forecasts of bioluminescent blooms.

RELATED PROJECTS

Initial development of the bathyphotometer was funded under the Office of Naval Research grant N00014-06-1-0153, entitled Bioluminescence Truth Data Measurement and Signature Detection. In collaboration with the Florida Fish and Wildlife Research Institute, ORCA is exploring the use of the bathyphotometer and Kilroy network described herein for detection of a toxic variety of the bioluminescent dinoflagellate *Pyrodinium bahamense*.

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HONORS/AWARDS/PRIZES

E.A. Widder, Ocean Research & Conservation Association named Environmentalist of the Year by the Conservation Alliance of St. Lucie County Florida May 2009

E.A. Widder, Ocean Research & Conservation Association named Blue Friend of the Year by the Loggerhead Marine Life Center Florida November 2009